

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: March 31, 1977

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Project Title: Organization and Expression of the Genome of Phage T4

Project No: G-32-A01

Project Director: Dr. Dwight H. Hall

Sponsor: DHEW/PHS/NIH - General Medical Sciences, Bethesda, MD

Agreement Period: From 3/1/77 Until 2/29/80 *
*Only the 01 year funded (3/1/77 - 2/28/78)

Type Agreement: Grant No. 7 R01 GM24455-01

Amount:
\$62,587 PHS
6,774 GIT (G-32-318)
\$69,361 TOTAL

Reports Required:
Annual Progress Reports with Continuation applications.
Terminal Progress Report upon grant expiration.

Sponsor Contact Person (s):

Technical Matters

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(thru OCA)

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Bethesda, MD 20014

Defense Priority Rating: None

Assigned to: Biology

(School/Laboratory)

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Project Code (GTRI)
Other

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT TERMINATION

Date: 3/20/78

Project Title: Organization and Expression of the Genome of Phage T4

Project No: G-32-A01

Project Director: Dr. D.H. Hall

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Effective Termination Date: 2/28/78 (End of 01 year)

Clearance of Accounting Charges: by 2/28/78

Grant/Contract Closeout Actions Remaining:

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☒ Other Annual Report of Expenditures due by 5/31/78.

NOTE: FOLLOW-ON (02 YEAR) IS G-32-A02.

Assigned to: Biology (School/Laboratory)

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Other _____

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →		GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT		GM24455-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)		PERIOD COVERED BY THIS REPORT	
Hall, Dwight H.		FROM	THROUGH
NAME OF ORGANIZATION		3/1/77	12/15/77
Georgia Institute of Technology			
TITLE (Repeat title shown in Item 1 on first page)			
Organization and Expression of the Genome of Phage T4			

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. (a) Dwight H. Hall, Kenneth Trofatter, and Diane L. Russell. Abstracts of Papers presented at the Bacteriophage Meeting, p. 99 (1977). Suppressors of Mutations in Gene 63 of Bacteriophage T4.
- (b) None
- (c) Lee A. Goscin, Gerard M. Collins, Glenn S. Rampe, and Dwight H. Hall. Manuscript in Preparation. Hydroxyurea-Sensitive Mutants of Bacteriophage T4. III. Mutants affecting Lysis.
2. Additions in professional personnel: L. Cheryl Miller
Ronald D. Snyder
3. The long-term goal is to reveal and explain how the genome of phage T4 is organized, why it is organized that way, and how the sequential expression of the genome is regulated. This is being approached by isolating and studying T4 mutants affecting genes that determine related enzymes, namely those involved in the biosynthesis of thymidylic acid in T4-infected Escherichia coli. The aims of the research are:
 - (a) to study the relation of these genes to each other and the regulation of their expression,
 - (b) to further characterize the genes in which mutants have been found, and
 - (c) to isolate and study mutants in more T4 genes controlling enzymes involved in the synthesis of thymidylate.

The goals set for the current year were:

- (a) to further characterize hus and far mutants of T4 genetically and biochemically, especially regulatory mutants such as hus13 and farP85,
- (b) to isolate more far mutants of different types and mutants resistant or sensitive to other drugs that affect pyrimidine metabolism, and
- (c) to study more false revertants of T4 mutants in gene 63, particularly looking for mutants in new genes.

We have continued to study our folate analog resistant mutant farP85 and other mutants in the same gene (mot). We have found that farP85 grows very poorly on a particular strain of Escherichia coli. This finding has helped us to identify and study mot mutants and shows that a normal mot gene is required for good viral growth on at least one host cell. We have shown that mutants (called sip) isolated by a very different method by Homyk and Weil at Vanderbilt University are also folate analog resistant mot mutants and that one of their suppressors (L₂) of a sip mutant decreases its resistance to folate analogs. (cont'd)

SECTION IV - SUMMARY PROGRESS REPORT (Continued)

These results suggest that L_2 affects another regulatory gene related to the mot gene.

In order to find new types of folate analog resistant (far) mutants, we have isolated far mutants at low temperature (30°C) and tested them for the ability to grow at high temperature (43°C) in the absence of folate analogs. Mutants that cannot grow at 43°C would define essential genes involved in regulation of gene expression and/or nucleotide metabolism and such mutants should be easier to study than previous far mutants. We have found several different mutants of this type in which the temperature-sensitivity and folate analog resistance seem to be caused by the same mutation. One of these mutants is near or in the mot gene and another is apparently in one of the genes coding for ribonucleoside diphosphate reductase.

More false revertants of mutants in gene 63 have been studied. These revertants have become more interesting with the recent finding from other laboratories that the gene 63 product is also an RNA ligase. Some of our results with these revertants are summarized in the following abstract of a talk given at the August, 1977 Bacteriophage Meeting at the Cold Spring Harbor Laboratories in New York.

SUPPRESSORS OF MUTATIONS IN GENE 63 OF BACTERIOPHAGE T4.

Dwight H. Hall, Kenneth Trofatter*, and Diane L. Russell*,
School of Biology, Georgia Institute of Technology, Atlanta,
Georgia, and *Department of Biochemistry, Duke University
Medical Center, Durham, North Carolina.

The protein product of T4 gene 63 catalyzes the attachment of tail fibers to fiberless phage particles (Wood & Henninger, J. Mol. Biol. 39: 603, 1969). The following observations suggest that the gene 63 product has a role in nucleotide metabolism: (1) on the genetic map of T4, gene 63 is close to several genes which code for enzymes involved in nucleotide metabolism; (2) the gene 63 product begins to be synthesized early after infection of E. coli (Wood & Henninger, 1969); and (3) although gene 63 appears to be nonessential for growth on many strains of E. coli, mutants in gene 63 fail to grow on E. coli OK305 when nucleotides are limiting.

In order to investigate whether the gene 63 product has a role in nucleotide metabolism, we have isolated false revertants of amM69 in gene 63. We screened for revertants that could grow on E. coli OK305 at 30°C but not at 43°C. These false revertants contain the original mutation in gene 63 and new suppressor mutations. Some of these suppressor mutations cause temperature sensitivity by themselves allowing single mutants carrying the suppressor to be recognized and isolated. The results of mapping and complementation studies indicate that most of these ts suppressors are in the t gene (lysis), one is in gene 5 (baseplate), and one is in gene 18 (sheath). The mutation in gene 18, (tsDH638), suppresses three different amber mutations in gene 63 but does not suppress amber mutations in several other genes.

None of the suppressors which have been characterized are in genes with known functions in nucleotide metabolism. However, an intriguing property of these false revertants is that they are very sensitive to hydroxyurea, an inhibitor of nucleotide metabolism.

SECTION IV - SUMMARY PROGRESS REPORT (Continued)

Our studies are providing new types of T4 mutants affecting related functions. Characterization of these mutants, some of which are regulatory, is leading to a better understanding of the organization and expression of the T4 genome.

A knowledge of the mechanisms involved in the control of phage development in infected bacteria should be helpful in the analysis of growth and development of higher organisms. It is conceivable that in some cases very similar mechanisms may be found. For example, it has been suggested that some of the enzymes involved in thymidylate synthesis are under coordinate control in mammalian cells just as they may be in T4-infected E. coli. An understanding of the phage genome may help in studying controlled and uncontrolled growth in higher organisms.

Some mutants are being recognized by their resistance or sensitivity to inhibitors of nucleotide metabolism, such as hydroxyurea and folate analogs, and are providing new insights into mechanisms of drug action and resistance.

The research goals for the coming year are:

- (a) to further characterize regulatory far and hus mutants genetically and biochemically, especially those that affect the mot gene,
- (b) to study our new temperature-sensitive far mutants, and
- (c) to understand the mechanism of suppression in our false revertants of mutants in gene 63 and its relation to the RNA ligase activity of the gene 63 product.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

12/20/77
Date

Principal Investigator or
Program Director